SRI PROJECT NO. LSU-4412

ENVIRONMENTAL FATE STUDIES OF HMX SCREENING STUDIES

Final Report, Phase I

By

Ronald J. Spanggord
William R. Mabey
Tsong-Wen Chou
Daniel L. Haynes
Philip L. Alferness
Doris S. Tse
Theodore Mill

December 1982

Supported By

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-82-C-2100

Katheryn Kenyon, Project Officer

SRI International
333 Ravenswood Avenue
Menlo Park, California, 94025

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

Environmental Fate Studies on HMX
Screening Studies

5. TYPE OF REPORT & PERIOD COVERED

Final Report, Phase I
May, 1982 - Nov, 1982

6. PERFORMING ORG. REPORT NUMBER

LSU-4412

7. AUTHOR(s)

Ronald J. Spanggord, W. R. Mabey, T. W. Chou, D. L. Haynes, P. L. Alferness, D. S. Tse, and T. Mill

8. CONTRACT OR GRANT NUMBER(s)

DAMD17-82-C-2100

9. PERFORMING ORGANIZATION NAME AND ADDRESS

SRI International
333 Ravenswood Avenue
Menlo Park, California 94025

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

62720A.3E162720A835.AA

11. CONTROLLING OFFICE NAME AND ADDRESS

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701
ATTN: SGRD-RMS

12. REPORT DATE

Dec, 1982

13. NO. OF PAGES

49

15. SECURITY CLASS. (of this report)

Unclassified

14. MONITORING AGENCY NAME & ADDRESS (if diff. from Controlling Office)

U.S. Army Medical Bioengineering R&D Laboratory
Environmental Protection Research Division
Fort Detrick, Frederick, Maryland 21701

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

N/A

16. DISTRIBUTION STATEMENT (of this report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

1,3,5,7-octahydro-1,3,5,7-tetranitrotetrazocine, HMX, sorption and biosorption partition coefficients, photochemical rate constant, biotransformation, abiotic reduction.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report describes preliminary screening test that were performed to identify the dominant transport and transformation processes that affect the persistence of 1,3,5,7-octahydro-1,3,5,7-tetranitrotetrazocine (HMX) in the aquatic environment.

The physical transport processes (volatilization, sediment sorption, and biosorption) were found not to be a major fate processes for HMX. A volatilization

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

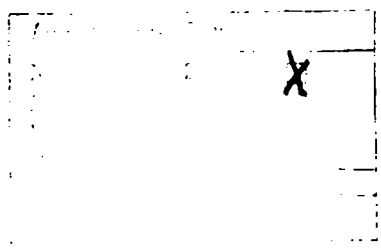
19. KEY WORDS (Continued)

20 ABSTRACT (Continued)

rate constant of ~~2.4~~ to 7.2 x ~~10~~$^{-4}$ day^{-1} ($t_{½}$ = 3000 to 1000 days) was estimated from laboratory experiments. The sorption partition coefficient, K_p, for HMX on Holston River sediment was measured at 8.7. Based on an organic carbon content of 1.3% in the sediment, a K_{oc} value of 670 was calculated. The biosorption partition coefficient, K_B, was measured to be 63.

Transformation processes that were identified to be important fate processes for HMX were photolysis and biotransformation. A first-order photolysis rate constant of 0.15 day^{-1} ($t_{½}$ = 5 days) was measured for the surface of Holston River water. The biotransformation of HMX occurs very slowly under anaerobic conditions in Holston River water but is greatly accelerated in the presence of extra organic nutrient. The aerobic biotransformation of HMX was found to occur rapidly in freshly collected HMX line wastestream water or in Holston River water with extra organic nutrients; however, the transforming organisms could not be enriched for the preliminary estimation of a biotransformation rate constant.

From laboratory studies and literature review, hydrolysis, oxidation, and abiotic reduction were considered not to be a major transformation processes for HMX.



A-1

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

EXECUTIVE SUMMARY

This report describes preliminary screening tests that were performed to identify the dominant transport and transformation processes that affect the persistence of 1,3,5,7-octahydro-1,3,5,7-tetranitro-tetrazocine (HMX) in the aquatic environment.

The physical transport processes (volatilization, sediment sorption, and biosorption) were found not to be major fate processes for HMX. A volatilization rate constant of 2.4 to 7.2 $\times 10^{-4}$ day^{-1} ($t_{1/2}$ = 3000 to 1000 days) was estimated from laboratory experiments. The sorption partition coefficient, K_p, for HMX on Holston River sediment was measured at 8.7. Based on an organic carbon content of 1.3% in the sediment, a K_{oc} value of 670 was calculated. The biosorption partition coefficient, K_B, was measured to be 63.

Transformation processes that were identified to be important fate processes for HMX were photolysis and biotransformation. A first-order photolysis rate constant of 0.15 day^{-1} ($t_{1/2}$ = 5 days) was measured for the surface of Holston River water. The biotransformation of HMX occurs very slowly under anaerobic conditions in Holston River water but is greatly accelerated in the presence of extra organic nutrient. The aerobic biotransformation of HMX was found to occur rapidly in freshly collected HMX line wastestream water or in Holston River water with extra organic nutrients; however, the transforming organisms could not be enriched for the preliminary estimation of a biotransformation rate constant.

From laboratory studies and literature review, hydrolysis, oxidation, and abiotic reduction were considered not to be major transformation processes for HMX.

ACKNOWLEDGEMENTS

We are deeply indebted to the technical staff of SRI International and their expertise in the areas encompassing this study. These staff include Ms. Doris S. Tse (chemical transformation) under the direction of Dr. William R. Mabey; Ms. Justine M. Whaley (biotransformation) under the direction of Dr. Tsong-Wen Chou; Mr. Daniel L. Haynes (physical transport); Mr. Philip L. Alferness (analytical chemistry) under the direction of Dr. Ronald J. Spanggord; and Dr. Theodore Mill for overall guidance and supervision.

I. INTRODUCTION

The production and manufacture of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and HMX-containing explosives have led to the generation of wastewaters that are eventually discharged into aquatic environments. The persistence of HMX in these environments and the dominant transport and transformation processes that control the loss and movement (fate) of HMX are unknown. An understanding of these processes is now recognized as an essential component of any overall risk assessment.

This report describes the Phase I efforts of SRI to identify processes that may affect the persistence of HMX in the aquatic environment. In this Phase, we reviewed the analytical methods for the determination of HMX and performed screening studies to estimate the importance of volatilization, sediment sorption, biosorption, photolysis, abiotic reduction, and aerobic and anaerobic biotransformation in controlling the fate of HMX. These screening studies were used as the basis for recommendations for detailed fate studies of dominant processes to be performed in Phase II as part of an overall environmental assessment.

II ANALYTICAL METHODS

Several chromatographic methods for the analysis of HMX have been reported. Glover and Hoffsommer (1973) described a thin-layer chromatographic (TLC) technique utilizing silica gel MF-254 TLC plates and development with a benzene/acetone (4/1) solvent system. Benzene extracts of water samples were analyzed and HMX was visualized by ultraviolet (UV) radiation at 254 nm. They found the detection limit to be 0.05 mg liter^{-1}.

Douse (1981) reported the use of fused silica capillary column gas chromatography with electron-capture detection as a method of analyzing for various explosives, including HMX. He stated that the detection limit is 100 pg. However, detection by electron capture requires complex sample clean-up procedures.

The problem of sample clean-up is eliminated with the use of the nitrosyl-specific thermal energy analyzer (TEA) as a detector for a high-performance liquid chromatographic (HPLC) system, as reported by Lafleur and Morriseau (1980). Using isooctane and ethanol as the mobile phase, along with a Lichrosorb SI-60 or Lichrosorb NH_3 column, they reported a detection limit of 1.0 ng. Because of the specificity of the TEA, large concentration factors can be attained by the extraction of water samples, with minimal interference from either extraction solvents or the sample itself.

Vouros et al. (1977) used chemical-ionization mass spectrometry in conjunction with an HPLC separation and collection system for the positive identification of HMX. They used NH_3 as the reagent gas for the ionization of HMX--rather than methane, water, hydrogen, or isobutane--because of the formation of the molecular ion at 314 m/e with NH_3.

We used a reverse-phase HPLC system for routine analysis of HMX in water and sediment samples. The following analysis conditions were applied to direct injection of water samples and of water and sediment

extracts. The analysis provides baseline separation of HMX, RDX, TNT, and related compounds, such as biodegradation metabolites and photolysis by-products that are routinely encountered. In general, reverse-phase HPLC was used to support all the investigations detailed in this study.

The reverse-phase HPLC analysis was performed with a Waters radial compression module with a C_{18} RCM cartridge. The mobile phase consisted of (a) H_2O and (b) 50% methanol/50% acetonitrile. A linear gradient was run from 35 to 55% (b) in 15 min, with a flow rate of 2.0 ml/min. Detection was by UV at 254 nm. The injection volume ranged from 50 to 400 µl, depending on the expected concentration of HMX. The internal standard (I.S.) method was used for quantitation, and a digital integrator was used to determine peak area.

We prepared water samples by adding an aliquot of the internal standard solution (3,5-dinitrotoluene in methanol) and filtering, if necessary. HMX standard solutions were run to determine response factors for sample quantitation. The detection limit for direct aqueous injections is 0.10 mg $liter^{-1}$. This limit can be lowered through an extraction and concentration step, as follows: A 1.0-liter water sample is extracted with dichloromethane (1 × 200 ml followed by 2 × 100 ml). The extracts are combined, dried over Na_2SO_4, evaporated to dryness in a roto-evaporator, and redissolved in a solution of 50% MeOH/50% H_2O.

We analyzed sediment samples for HMX and other munitions-related compounds by extraction with ethyl acetate in the following manner. Wet sediment (10-15 g) was combined with 10 ml of ethyl acetate in a 50-ml, screw-cap centrifuge tube and shaken for 2-3 min. Then the sample was centrifuged for 10 min at 3000 rpm, and the ethyl acetate was removed by pipet. This procedure was repeated twice. The sediment was then acidified with 5.0 ml of 2N HCl and extracted three more times. The extracts were combined and, depending on the expected concentration of HMX, either concentrated or diluted. A 1.0-µl aliquot of the prepared extract was evaporated under a stream of N_2 and redissolved in 1.0 ml of the internal standard solution and 1.0 ml of H_2O. The detection limit for HMX, without clean-up procedures, is 0.5 µg g^{-1}.

A normal-phase HPLC system was developed for use in conjunction with the TEA. In this system, a Waters silica RCM cartridge was used with a mobile-phase system of 4% methanol in dichloromethane at a flow rate of 2.0 ml/min. The TEA conditions were as follows: pyrolyzer temperature, 550°C; carrier gas, argon at 20 ml/min; oxygen flow, 30 ml/min; and vacuum, 0.5-1.0 torr. These conditions resulted in a detection limit of 8 ng of injected material.

Thus, judging from data available in the literature and the results of studies performed in our laboratory, highly selective and sensitive analytical techniques are available for the determination of HMX in fate assessment studies.

III. PHYSICAL TRANSPORT

Several physical transport processes were investigated in this study. They were the solubility of HMX in water, volatilization of HMX from water, and adsorption of HMX on sediment collected from the Holston River.

A. Solubility in Water

The solubility of HMX in water at 10, 20, and 30°C was measured by the method of May and Wasik (1978), and the results are presented in Table 1.

Table 1

WATER SOLUBILITY OF HMX AT 10, 20 AND 30°C

Temperature (°C)	Solubility of HMX (mg liter^{-1})
10	1.21 ± 0.04
20	2.63 ± 0.01
30	5.7 ± 0.1

Barkley (1977) reported the solubility of HMX in water at 20°C to be 6.6 mg l^{-1}. This is somewhat higher than our reported value in Table 1. However, our experience with the method of May and Wasik has shown that the measured solubility values obtained thereby are lower than those obtained by traditional solubility methods. This is probably because of saturation method used by May and Wasik, in which a large surface area of HMX is exposed to the water while avoiding the presence of suspended HMX in the solution. This method avoids the sample-handling problems (filtration, centrifugation), and the reproducibility of data obtained by this method has been found to be superior to that in more traditional methods (Campbell, 1930).

B. Volatilization from Water

The volatilization rate of a chemical dissolved in water is a first-order process (Smith et al., 1980, 1981) that can be mathematically described, as shown in Equations 1 and 2:

$$\frac{-dC}{dt} = k_v C \qquad (1)$$

$$k_v = \frac{1}{L}\left[\frac{1}{k_l^c} + \frac{RT}{Hk_g^c}\right]^{-1} \qquad (2)$$

where

- C = concentration of chemical in water (moles liter^{-1})
- k_v = volatilization rate constant (hours^{-1})
- L = solution depth (cm)
- k_l^c = liquid-phase mass transfer coefficient (cm hr^{-1}) of chemical C
- H = Henry's constant (torr liter mole^{-1})
- k_g^c = gas-phase mass transfer coefficient (cm hr^{-1}) of chemical C
- R = gas constant (62.3 liter torr mole^{-1} deg^{-1})
- T = temperature (deg, Kelvin)

Equation (2) can be simplified, depending on the magnitude of Henry's constant. If H is greater than 3500 torr M^{-1}, then the second term of Equation (2) is small compared with the first, and liquid-phase mass-transport resistance determines the volatilization rate. Similarly, if H is less than 10 torr M^{-1}, then the first term of Equation (2) is small compared with the second, and gas-phase mass-transport resistance controls the volatilization rate. Both terms are important if H is between 3500 and 10 torr M^{-1}.

We measured Henry's constant for HMX by the method of Mackay et al. (1979). In this method, a solution of known volume and HMX concentration is purged with an inert gas. The change in HMX concentration is monitored as a function of the purge gas, according to Equation 3.

$$\ln\left(\frac{C_o}{C}\right) = \frac{H}{\upsilon RT}(V) \qquad (3)$$

where

C = HMX concentration at a given purge gas volume

C_o = initial HMX concentration

H = Henry's constant

υ = volume of solution

V = purge gas volume

A plot of ln C/C_o versus V yields a straight line with a slope of $H/\upsilon RT$.

After applying this method using 7500 liters of purge gas, no change in the HMX concentration was noted. Although Henry's constant could not be measured, an upper limit of $H \leq 0.15$ torr M^{-1} was estimated. The magnitude of H suggests that volatilization of HMX is controlled by mass-transport resistence in the gas phase.

The volatilization rate constant (Equation 2) can now be reduced to Equation 4.

$$k_v = \frac{1}{L}\left[\frac{RT}{Hk_g^c}\right]^{-1} \qquad (4)$$

The gas-phase mass-transport resistance coefficient for HMX (k_g^c) can be estimated from Equation 5, which states that the ratio of the gas-phase mass-transport resistance coefficient of HMX and water is approximately equal to the square root of the molecular weights of water and HMX (Liss and Slater, 1974).

$$\frac{k_g^c}{k_g^w} = \left(\frac{18}{296}\right)^{1/2} = 0.25 \qquad (5)$$

Liss and Slater estimated k_g^w to be 1000 to 3000 cm hr^{-1} for water. Therefore, k_g^c for HMX can be estimated to range from 250 to 750 cm hr^{-1}.

Using the estimated values of k_g^c and H, the volatilization rate constant in a 200-cm-deep water body is approximately 7.2 to 2.4 $\times 10^{-4}$ day^{-1}. These values corresponds to half-lives of 1000 to 3000 days and suggest that volatilization will not be an important fate process for HMX.

C. Adsorption by Sediments

Adsorption of organic solutes into sediments and biota can be a very important process in the aquatic environment. The sediments can act as sinks for adsorbed materials, removing them from the water column. However, because the sorbate can also be released (desorbed) from the sediments later, contaminated sediments can also be a source of pollution.

Adsorption is usually described by the Freundlich adsorption isotherm, which is an empirical relationship (Equation 6):

$$C_s = K_p C_w^{1/n} \qquad (6)$$

where C_s is the sediment concentration of the chemical, K_p is the partition coefficient, C_w is the aqueous concentration of the chemical, and n is a constant. At low solute concentrations, n ≈ 1, and Equation 6 reduces to (Equation 7):

$$K_p = \frac{C_s}{C_w} = \frac{(\mu g\ g^{-1})}{(\mu g\ ml^{-1})} = F_{oc}\ K_{oc} \qquad (7)$$

where F_{oc} is the fraction of organic carbon in the sediment and K_{oc} is the adsorption partition coefficient normalized for the sediment organic content. The screening study consists of estimating K_{oc} from the solubility of the chemical in water and measuring K_p and K_{oc} at one concentration of chemical and two sediment loadings.

Karickhoff et al. (1974) showed that:

$$\text{Log } K_{oc} = -0.921 \log X_s - 0.00953(MP - 25) \quad (8)$$

where X_s is the solubility in mole fractions and MP is the melting point (greater than 25°C) in °C. Using a solubility for HMX of 2.6 mg liter^{-1} (8.8×10^{-6} M), K_{oc} is equal to 234. If $F_{oc} = 0.013$, then K_p would be about 3. This value serves as a preliminary estimate of the magnitude of K_p.

A screening adsorption isotherm was measured for HMX on Holston River sediment according to previously developed laboratory protocols (Mill et. al., 1980). Sediment from an upstream site was used for the isotherm. Both supernatant and sediment were analyzed for their HMX content. The results of the isotherm are presented in Table 2.

Sample 1 contained water only; Sample 5 contained sediment and water. No interferences were noted in the analysis of these blanks. No HMX was found in the sediment extracts, although losses were found when the total amounts of HMX in the supernatants were compared with starting amounts (e.g., Sample 2). The maximum HMX absorbed was calculated by the difference between that in Sample 2 and the supernatant amount in each sample.

This isotherm was remeasured to account for the absence of HMX in the sediment extracts. Formaldehyde was added to determine whether biodegradation was responsible for losses of HMX. Also, the entire sediment from each sample was extracted and analyzed rather than the sediment from 10-ml aliquots of the isotherm mixture, which was the procedure for the first isotherm. Results of those analyses are presented in Table 3. The results on Sample 1 (formaldehyde added) and

Table 2

FIRST HMX SCREENING ADSORPTION ISOTHERM

Sample #	Supernatant Conc. of HMX (C_w)	Total HMX Supernatant (µg)	Maximum HMX Adsorbed (µg)	Sediment Dry Wt (g)	C_s (µg/g)
1	--	--	--	--	--
2	1.85	185	--	--	--
3	1.58	158	27	2.1	12.9
4	1.54	154	31	2.2	14.1
5	--	--	--	7.0	--
6	1.27	127	58	6.3	9.2
	1.18	118	67	6.3	10.6
7	1.14	114	71	6.6	10.8
	1.14	114	71	6.6	10.8

Table 3

SECOND HMX ADSORPTION ISOTHERM

Sample	Concentration ($\mu g\ ml^{-1}$)	HMX (μg)	Sediment (dry, g)	C_e ($\mu g\ g^{-1}$)	K_p ($\mu g\ g^{-1}\ \mu g^{-1}\ ml$)	K_{oc} ($\mu g\ g^{-1}\ \mu g^{-1}\ ml$)	HMX Taken (μg)	HMX Found (μg)	% Recovery
1-supernatant	1.05	108	7	—	4.4 (8.6)	340(650)	171	140	82
2-supernatant	1.09	112	7	--	4.5 (7.7)	340(690)	171	146	85
3-supernatant	1.71	171	--	—	—	—	171	171	100
1-sediment	1.29 (in 25 ml)	32	7	4.6(9.0)	--	—	—	—	--
2-sediment	1.35 (in 25 ml)	34	7	4.9(8.4)	—	—	—	—	—

Notes: Values in parentheses are calculated using supernatant data only. Supernatant Samples 1 and 3 contained formaldehyde.

Sample 2 (no formaldehyde added) did not differ significantly, indicating that HMX did not appreciably biodegrade in the sediment, which is confirmed by the presence of HMX in the sediment extracts of the second isotherm. This suggests that the amount of HMX extracted from 10% of the sediment of the first isotherm was just too low to be measured.

Although some HMX was recovered from the sediment in the second isotherm about 15-18% of the total HMX added to Samples 1 and 2 was not recovered. A fourth extract of the sediment from Sample 1 showed that a small, but unmeasurable, amount of HMX may still be in the sediment after the initial extraction. This unextracted HMX, plus the uncertainty in the analysis of the sediment extract, could account for the low recovery.

The partition coefficient, K_p, was calculated from a linear least squares plot of C_w versus C_s. An upper limit of $K_p \leq 8.7$ was calculated using values of C_s determined from the difference between the amount of HMX added and that found in the supernatant. The fractional organics content, F_{oc}, of the sediment was measured and found to be 0.013. From Equation 7 a value of $K_{oc} \leq 670$ was calculated. These values of K_p and K_{oc} indicate that sediment sorption will not be a major fate for HMX unless other fate processes are slow.

IV. CHEMICAL TRANSFORMATION

A. Photochemistry

1. Ultraviolet Absorption Spectrum

The UV adsorption spectrum of HMX in water containing 10% acetonitrile (Figure 1) was obtained using a HP 8450 A spectrophotometer. The molar extinction coefficients (ε_λ) for wavelengths in the solar spectral region ($\lambda > 298$ nm) were calculated using the Beer-Lambert Law. They are shown in Table 4.

Because RDX is structurally related to HMX, we obtained the UV spectrum (Figure 2) and calculated ε_λ values (Table 5) for RDX for comparative purposes.

We also measured the UV spectra of unfiltered and filtered Holston River water samples obtained from above (HU) and below (HD) the HMX line wastestream outfall (Figures 3 and 4). These data show that the downstream water has a higher absorbance than the upstream water in the spectral region below 300 nm and that the two water samples have similar low absorbances in the solar region above 300 nm.

2. Photolysis Rate Constant in Sunlight

We estimated the HMX photolysis rate constant (k_{PE}) from outdoor sunlight experiments using 0.5-ppm solutions of HMX in pure and filtered Holston River water in tightly capped borosilicate tubes in conjunction with p-nitroacetophenone/pyridine (PNAP/PYR) actinometer solutions. The photolysis of HMX followed first-order kinetics and from the measured rate constants, we estimated a half-life for HMX of 4-5 days. Rate constants listed in Table 6 are corrected to flat-surface water values.

Figure 1

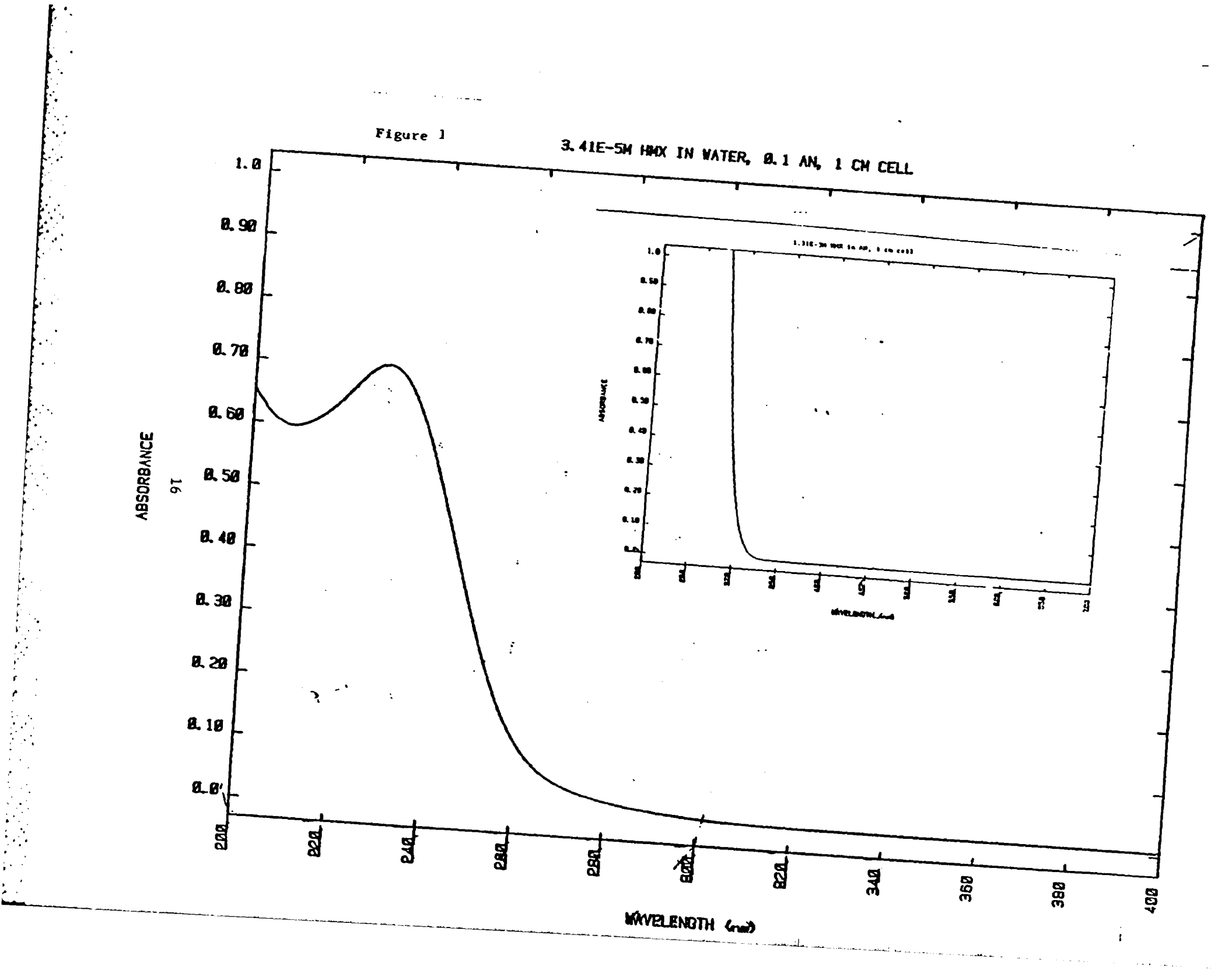

3.41E-5M HMX IN WATER, 0.1 AN, 1 CM CELL
ABSORBANCE
1.0
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
200
220
240
260
280
300
320
340
360
380
400
WAVELENGTH (nm)

Table 4

EXTINCTION COEFFICIENTS OF HMX SOLUTION AT VARIOUS WAVELENGTHS

ε_λ M^{-1} cm^{-1}

λnm	ε_λ [a]	ε_λ [b]	avg ε_λ
299	160	170	165
304	100	100	100
309	66	60	63
314	42	33	38
319	28	17	23
323	22	10	16
340	15	2	8
370	13	1	7
400	11	0	6
430	2	0	1
460	1	0	0
494	1	0	0

---

[a] Calculated from spectrum of 1.31×10^{-4} M HMX in 10% acetonitrile (AN) in 10 cm cell.

[b] Calculated from spectrum of 1.31×10^{-3} M HMX in AN in a 1-cm cell.

Figure 2

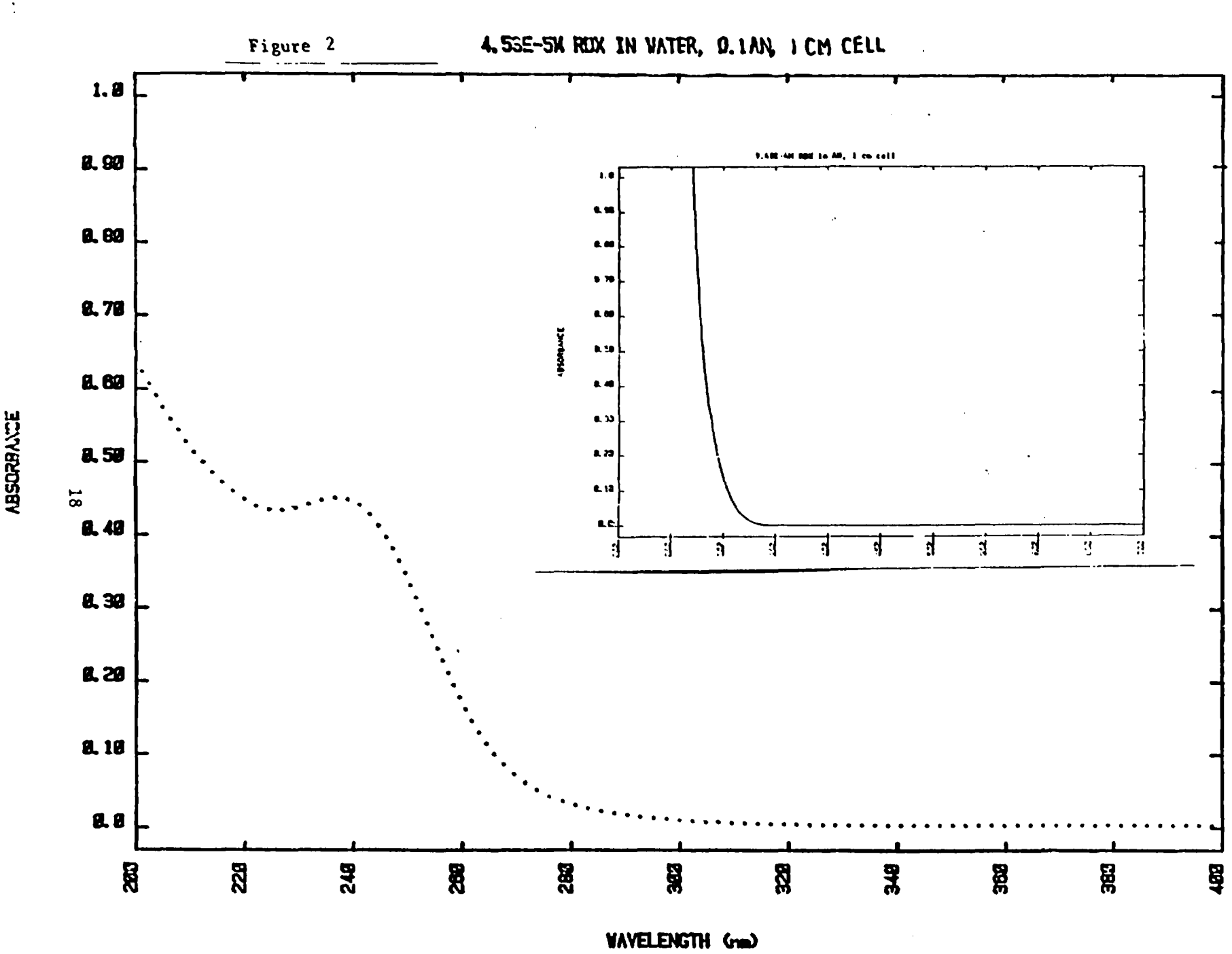

4.55E-5M RDX IN WATER, 0.1AN, 1 CM CELL
ABSORBANCE
1.0
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
200
220
240
260
280
300
320
340
360
380
400
WAVELENGTH (nm)

Table 5

EXTINCTION COEFFICIENTS OF RDX SOLUTION AT VARIOUS WAVELENGTHS

$\varepsilon_\lambda M^{-1} cm^{-1}$

λ nm	ε_λ [a]	ε_λ [b]	avg ε_λ
299	170	157	164
304	116	108	112
309	78	72	75
314	50	47	49
319	32	29	31
323	22	17	20
340	8	3	6
370	6	0	3
400	0	0	0
430	0	0	0
460	0	0	0

[a]Calculated from spectrum of 1.90×10^{-4} M RDX in 10% AN in a 1-cm cell.

[b]Calculated from spectrum of 9.48×10^{-4} M RDX in AN in a 1-cm cell.

Figure 3

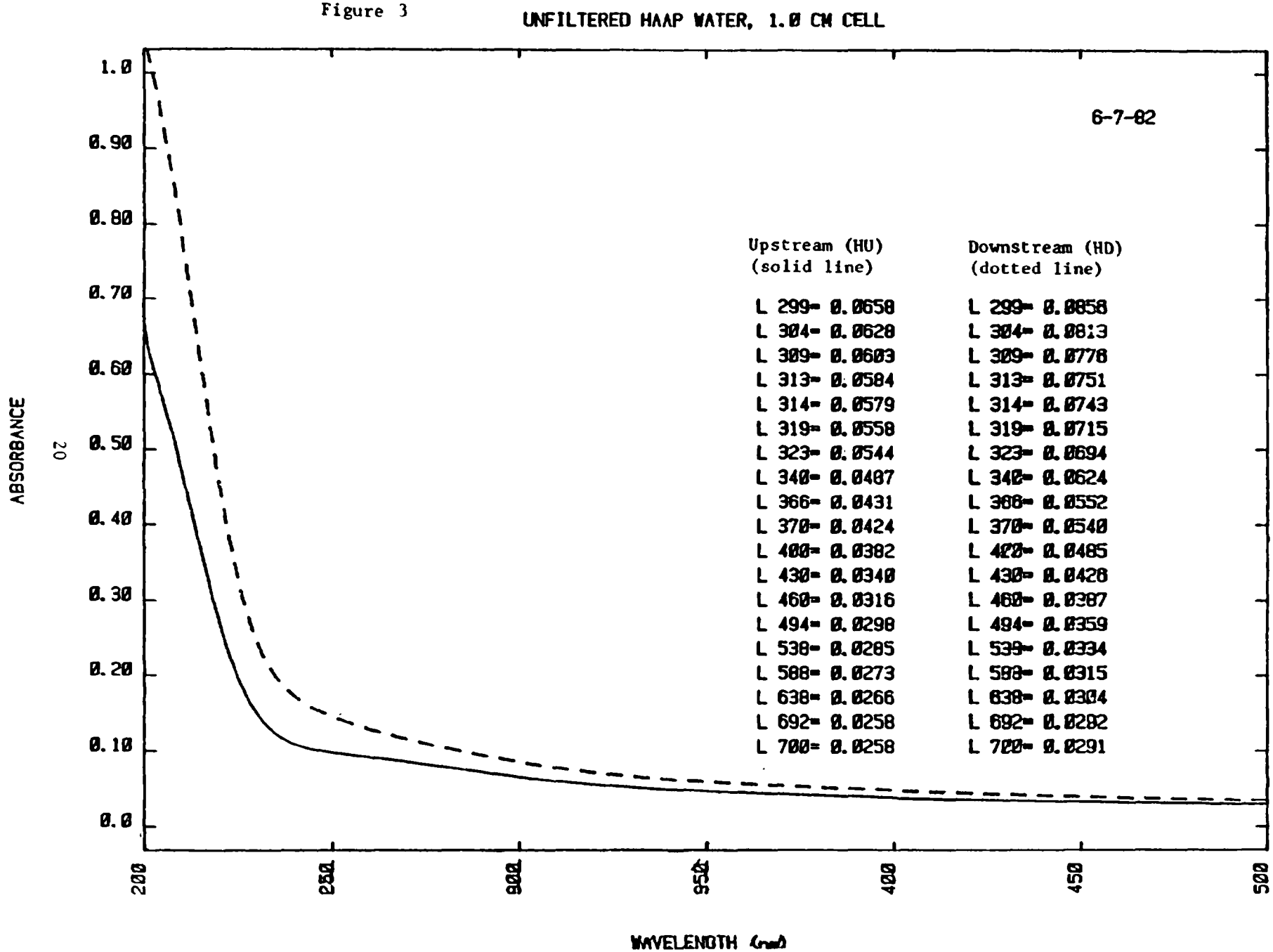

UNFILTERED HAAP WATER, 1.0 CM CELL
6-7-82
Upstream (HU)
(solid line)
L 299= 0.0658
L 304= 0.0628
L 309= 0.0603
L 313= 0.0584
L 314= 0.0579
L 319= 0.0558
L 323= 0.0544
L 340= 0.0487
L 366= 0.0431
L 370= 0.0424
L 400= 0.0382
L 430= 0.0340
L 460= 0.0316
L 494= 0.0290
L 530= 0.0285
L 588= 0.0273
L 638= 0.0266
L 692= 0.0258
L 700= 0.0258
Downstream (HD)
(dotted line)
L 299= 0.0858
L 304= 0.0813
L 309= 0.0778
L 313= 0.0751
L 314= 0.0743
L 319= 0.0715
L 323= 0.0694
L 340= 0.0624
L 366= 0.0552
L 370= 0.0540
L 400= 0.0485
L 430= 0.0428
L 460= 0.0387
L 494= 0.0359
L 530= 0.0334
L 588= 0.0315
L 638= 0.0304
L 692= 0.0282
L 700= 0.0291
ABSORBANCE
1.0
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
200
250
300
350
400
450
500
WAVELENGTH (nm)

Figure 4

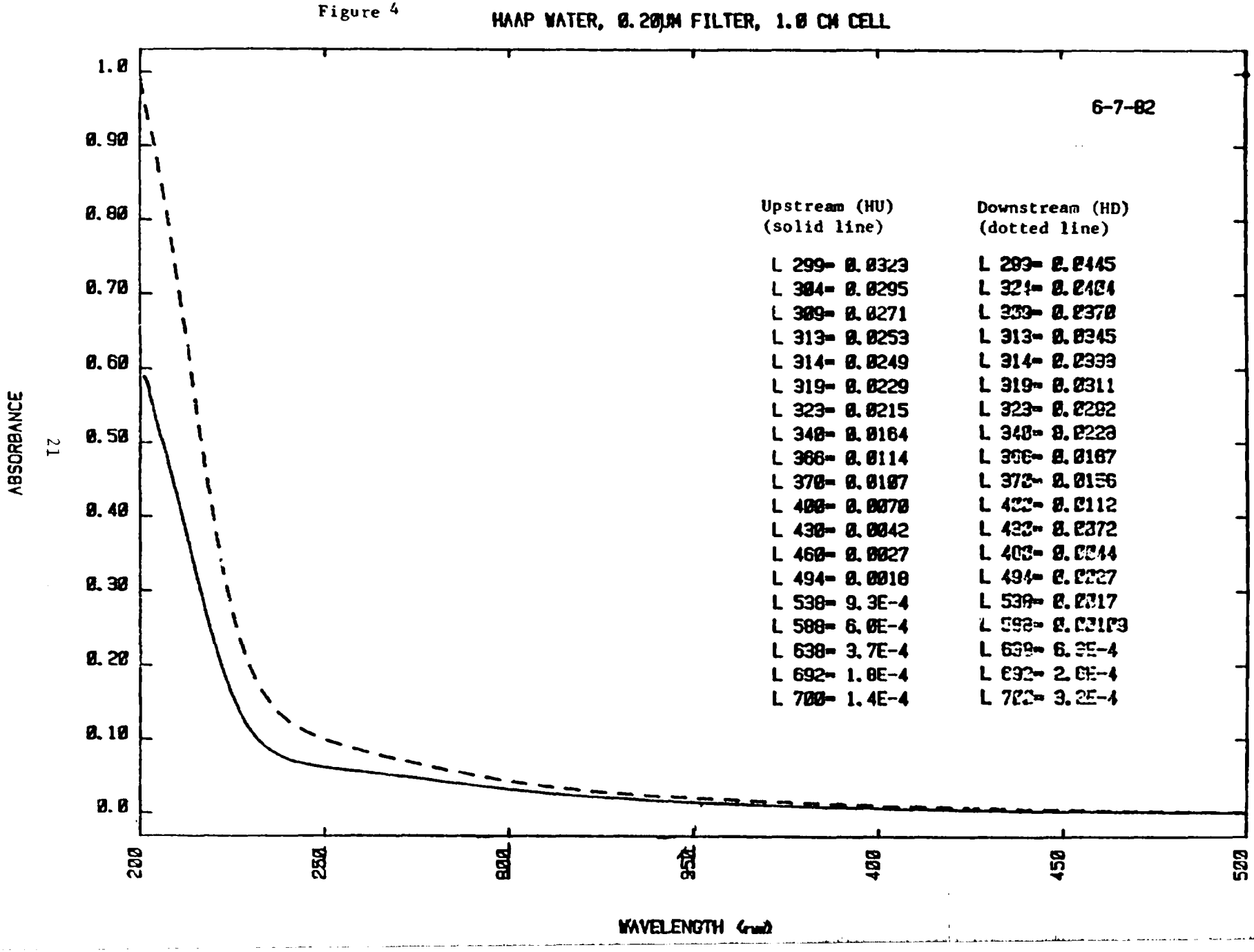

HAAP WATER, 0.20µM FILTER, 1.0 CM CELL
6-7-82
Upstream (HU)
(solid line)
L 299= 0.0323
L 304= 0.0295
L 309= 0.0271
L 313= 0.0253
L 314= 0.0249
L 319= 0.0229
L 323= 0.0215
L 340= 0.0164
L 366= 0.0114
L 370= 0.0107
L 400= 0.0070
L 430= 0.0042
L 460= 0.0027
L 494= 0.0018
L 530= 9.3E-4
L 580= 6.0E-4
L 630= 3.7E-4
L 692= 1.0E-4
L 700= 1.4E-4
Downstream (HD)
(dotted line)
L 299= 0.0445
L 324= 0.0404
L 339= 0.0370
L 313= 0.0345
L 314= 0.0333
L 319= 0.0311
L 323= 0.0292
L 340= 0.0228
L 366= 0.0167
L 372= 0.0156
L 422= 0.0112
L 432= 0.0072
L 460= 0.0044
L 494= 0.0027
L 530= 0.0017
L 598= 0.00103
L 639= 6.3E-4
L 692= 2.0E-4
L 722= 3.2E-4
1.0
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
ABSORBANCE
200
250
300
350
400
450
500
WAVELENGTH (nm)

Table 6

SUNLIGHT PHOTOLYSIS RATE CONSTANTS[a] FOR 0.50 ppm HMX[b] IN PURE AND HOLSTON RIVER WATER IN LATE SPRING

	5 PM Data		11 AM Data		
Sample	$k_p' \times 10$ (d^{-1})[c]	$t_{1/2}$ (d)[c]	$k_p \times 10$ (d^{-1})[c]	$t_{1/2}$(d)[c]	k_p'/k_p
PNAP/PYR[d]	1.51 ± 0.03	4.60	1.59 ± 0.02	4.35	0.95
HMX/PW[e]	1.38 ± 0.01	5.02	1.60 ± 0.04	4.32	0.86
HMX/HU[e]	1.48 ± 0.02	4.68	1.66 ± 0.03	4.17	0.89
HMX/HD[e,f]	1.50 ± 0.02	4.62	1.69 ± 0.04	4.11	0.89

[a]Corrected for open dish. Rate constants for HMX were 2.5 times greater when measured in borosilicate tubes than when measured in dishes.

[b]0.50 ppm HMX = 1.70×10^{-6} M.

[c]In terms of 24-hour day.

[d]PNAP = 1.00×10^{-5} M; PYR = 0.05 M; $\phi = 8.45 \times 10^{-4}$.

[e]PW = pure water; HU = Holston upstream; HD = Holston downstream.

[f]0.16 ppm of HMX was already present in the HD water before 0.50 ppm of HMX was added for the photolysis experiment.

For each solution, both first-order kinetic plots have good correlation coefficients ($r^2 > 0.997$), but the values of k_p for HMX for the 11 AM series were 10% higher than for the 5 PM series; values for PNAP/PYR actinometer were almost unchanged.

Our data on the photolysis of HMX in HU and HD waters show that humic substances or other chemicals from the wastestream do not significantly affect the photolysis rates of HMX.

3. Quantum Yield

With optically thin solutions, the quantum yield, ϕ_C, of a chemical (C) and an actinometer (A) can be expressed in terms of the day-averaged sunlight flux, L_λ, in the wavelength interval λ; the measured sunlight photolysis rate constants, k_{pC} and k_{pA}; and the extinction coefficients at wavelengths λ, $\varepsilon_{\lambda C}$ and $\varepsilon_{\lambda A}$ (Equation 9):

$$\phi_C = \frac{k_{pC}}{k_{pA}} \cdot \frac{\Sigma L_\lambda \varepsilon_{\lambda A}}{\Sigma L_\lambda \varepsilon_{\lambda C}} \cdot \phi_A \qquad (9)$$

Sunlight intensity fluxes for HMX and for the actinometer appear in Table 7.

In a June 15-22 experiment we measured the loss of HMX and of PNAP/PYR* in tubes co-irradiated for several days and plotted $\ln(C_o/C_t)$ versus $\ln(A_o/A_t)$ to obtain k_{pC}/k_{pA}, which was found to be 0.979. Thus, substituting this value of k_{pC}/k_{pA} and 8.45×10^{-4}* for the quantum yield of the actinometer, the data from Table 7, and the interpolated L_λ values for June 15-22)† into Equation 9, we calculated the quantum yield of HMX

---

*1.0×10^{-5} M PNAP/0.05 M PYR actinometer; ϕ = 0.0169 [PYR].

†Interpolated from the L_g values given for April 16 and July 24 in Laboratory Protocols for Evaluating the Fate of Organic Chemicals in Air and Water, Final Draft SRI Contract No. 68-03-2227, Sept. 1981, p. 60.

Table 7

SUNLIGHT INTENSITY FLUX FOR HMX (C) AND PNAP (A)

λnm	$\varepsilon_{\lambda A}$ [a]	$\varepsilon_{\lambda C}$ [b]	L_λ [c]	$L_\lambda \varepsilon_{\lambda A}$	$L_\lambda \varepsilon_{\lambda C}$
299	3100	165	2.00 (-4)	6.20 (-1)	3.30 (-2)
304	2600	100	1.98 (-3)	5.15	1.98 (-1)
309	2200	63	6.17 (-3)	13.6	3.89 (-1)
314	2000	38	1.45 (-2)	29.0	5.51 (-1)
319	1500	23	2.37 (-2)	35.6	5.45 (-1)
323	1100	16	2.42 (-2)	26.6	3.87 (-1)
340	470	8	2.92 (-1)	137	2.34
370	71	7	3.77 (-1)	26.8	2.64
400	0	6	8.14 (-1)	0	4.88
430	0	1	1.07	0	1.07
460	0	0	1.24	0	0
494	0	0	1.72	0	0
	$\Sigma L_\lambda \varepsilon_\lambda$			274	13.0

---

[a]Calculated from spectrum of 6.6×10^{-5}M PNAP in 10% AN in 1-cm cell.

[b]From Table 4.

[c]Interpolated from L_λ values of April 16 and July 24 at latitude 40°N.

in pure water to be 0.017. This value is an order of magnitude smaller than that of RDX (0.16, Spanggord, et al. 1980). Substituting the HMX ϕ value into Equations 10 and 11, we calculated the sunlight, clear-sky photolysis rate constants for HMX and the actinometer to be 0.22 d^{-1} and 0.23 d^{-1}, respectively. These values are almost twice as large as our observed rate constants of 0.14 d^{-1} and 0.15 d^{-1} for HMX and PNAP/PYR. The smaller observed rate constants probably are due to the foggy sky conditions during the photolysis experiment.

$$K_{pCE} = \phi_C \Sigma L_\lambda \epsilon_{\lambda C} \qquad (10)$$

$$K_{pAE} = \phi_A \Sigma L_\lambda \epsilon_{\lambda A} \qquad (11)$$

From these results, we conclude that photolysis of HMX is an important chemical transformation process and that more detailed study of the process including product formation is needed.

B. Chemical Reduction

We performed several experiments designed to test whether reduction by chemical processes may be a significant process in anaerobic sediments. These experiments, using ferriprotoporphyrin and ferrous ion dithionite, and bisulfide ions with 1×10^{-5} M HMX, indicate that HMX is resistant to reduction, but that reduction by dithionite ion is promoted slightly by increasing pH. These experiments are based on the premise that both ferrous ion and sulfur can act as 1-electron reductants for nitro and halogen compounds if oxygen is excluded from competition.

All the solutions used in the experiments were purged with argon to exclude oxygen. With a 5-fold excess of $FeSO_4$ (pH 4.8), we observed no reduction (<3%) of HMX after 380 hours. When the ferrous sulfate concentration was increased to a 50-fold excess (pH 7.8), the amount of reduction increased only slightly (8%) after 310 hours.

Reduced hematin, which is an iron(II) protoporphyrin system, is reported to be a good reducing agent for chloronitro compounds (Khalifa et al., 1976). Commercially available hematin, obtained as the ferriprotoporphyrin hydroxide from bovine blood (Sigma Chemical Company), was first reduced with a 6-fold excess of sodium dithionite, $Na_2S_2O_4$, under argon in a basic carbonate solution before being mixed with HMX solution (pH 11.2). We observed no reduction of HMX (<2%) after seven days under these conditions. However, there was a 30% reduction of HMX in the control solution with dithionite at pH 11.2 but with no hematin. An experiment without hematin was repeated with a 20-fold excess of sodium dithionite at two pH values for seven days; again, there was a 30% reduction of HMX at pH 11.2 but only 10% reduction at pH 6.8. Thus, these experiments show that Fe^{2+} and reduced hematin are not very effective reducing agents for HMX, but that dithionite is somewhat effective.

We also performed some experiments with bilsulfited ion, HS^-, because its reduction potential lies between those of Fe^{2+} and $S_2O_4^{2-}$ [$E°$ (HS^-) = 0.478; $E°$ (Fe^{2+}) = -0.770, and $E°$ ($S_2O_4^{2-}$) = 1.12 *]. Sulfide ion is also a good reducing agent, but its concentration in environmental sediments must be low because of the high pK_a for HS^- (12), whereas the pK_a for H_2S is 7.0. Therefore, a significant concentration of HS^- can be present in sediments.

We conducted experiments with 1.1×10^{-5} M HMX and a 28-fold excess (2.9×10^{-4} M) of ammonium sulfide at pH 8.3 where HS^- is the dominant species in solution. These experiments were performed with and without oxygen, with the former being the control. Aliquots of these solutions were removed at various time intervals; each was quenched with 20 µl of 0.1 M HCl solution (final solution with pH 3) and was analyzed immediately by HPLC. We observed no reduction of HMX (<2%) after eight days under both aerobic and anaerobic conditions. Thus we conclude that bisulfide is not an efficient reducing agent for HMX. All of the experimental results are summarized in Table 8.

---

*CRC Handbook of Chemistry and Physics, 50th edition, p. D-109.

Table 8

REDUCTION OF AQUEOUS HMX SOLUTIONS WITH VARIOUS REDUCING AGENTS[a]

Reducing Agent	Concentration ($M \times 10^5$)	pH of Solution	Reaction Period, H	% Reductions
$FeSO_4$	4.8	4.8	380	<3
$FeSO_4$	46	7.8	310	8
Reduced hematin	5.3	11.2	168	<2
$Na_2S_2O_4$	13	11.2	168	30
$Na_2S_2O_4$[b]	18	11.1	168	33
HS^-	29	8.3	192	2

[a] HMX = 9.9×10^{-6} M unless otherwise noted.

[b] HMX = 1.1×10^{-5} M.

Of the various substances investigated (ferriprotoporphyrin and ferrous, dithionite, and bisulfide ions), dithionite ion was the only effective reducing agent (30% reduction after seven days at pH 11). However, dithionite ion is not an environmentally relevant reducing agent because it is likely to be oxidized by most oxidizing agents present in the environment, such as Fe^{3+} and Hg^{2+}. Therefore, chemical reduction of HMX in anaerobic environments does not appear to be an important process.

C. Hydrolysis of HMX

There are only a few reports of the hydrolysis of HMX; however, because of expected similarities in the chemistries of RDX and HMX, we believe that hydrolysis of HMX is not an important process compared to photolysis.

Hydrolysis of RDX has been studied by Epstein and Winkler (1951), Jones (1954), Hoffsommer and Kubose (1977), and Sikka et al. (1980). They all observed that alkaline hydrolysis of RDX follows second-order kinetics and that the process is very slow below 30°C. The rate constants reported cannot be compared because of the different reaction conditions (solvent, pH, and temperature) used. However, under the same hydrolysis conditions, HMX is reported to have a hydrolysis rate constant at least two orders of magnitude smaller than that of RDX. At pH 8 and 15°C, the half-lives of RDX and HMX in aqueous acetone solutions are 22 days and 21 years, respectively.

Therefore, if hydrolysis of HMX occurs, it does so only to a very small extent under environmental conditions.

D. Oxidation of HMX

Because the alkyl C-H bonds in HMX are not readily oxidized under environmental conditions (Mill et al, 1980), and because the $N-NO_2$ groups are already highly oxidized, we believe that oxidation of HMX is not an important process.

V. BIOTRANSFORMATION

This investigation was performed to determine whether microorganisms could effect the transformation of HMX in aquatic environments.

HMX is structurally similar to RDX and therefore its biotransformation may follow a similar pattern. Previous studies on the biotransformation of RDX (Spanggord et al., 1980) showed that RDX was not readily biotransformed in water under aerobic conditions. It was, however, biotransformed slowly in the presence of 1% Holston River sediment after the lag period. Sikka et al. (1980) observed the same phenomenon, and they also reported the evolution of $^{14}CO_2$ from ^{14}C-RDX in the water with HMX-production-line wastewater sediment.

We found that RDX was readily biotransformed under anaerobic conditions with small amounts of extra organic nutrients (30 ppm yeast extract). In transferring flasks containing 10 ppm RDX and 30 ppm yeast extract, RDX disappeared in two to three days. McCormick et al. (1981) confirmed our findings and reported the metabolites to be mono-, di-, and trinitroso derivatives of RDX, hydrazine, dimethyl hydrazines, formaldehyde, and methanol.

A. Biotransformation Screening

Screening tests for the aerobic and anaerobic biotransformation of HMX were conducted with Holston River water that had been collected downstream of the HMX wasteline. The water was mixed well and screened through a cloth filter. Bottom sediments were collected from HMX wasteline and were mixed and screened through a 2-mm sieve.

For the aerobic studies, 2-liter samples containing (1) water alone, (2) water plus 50 ppm sterile yeast extract, and (3) water plus 1% dry weight of bottom sediment from the HMX wastelines were placed in 4-liter bottles, and 4 ppm HMX was added to each sample. The pH was maintained

between 7 and 8 by bubbling air into the bottles to reduce the dissolved CO_2. All the waters were incubated at 20 to 25°C in the dark. The bottles were shaken several times a week and at every sampling.

For the anaerobic studies, 1 liter of each of the above solutions (1-3) was placed in 1-liter Erlenmeyer flasks and bubbled with N_2 gas. The flask was then sealed with a rubber stopper. Purging with N_2 was repeated after each sampling. The pH was kept between 7 and 8; it was raised by N_2 purging (to remove CO_2) and lowered by CO_2 addition. These solutions were also incubated at 20 to 25°C in the dark. The contents of the flasks were mixed with a magnetic stirrer and bubbled with N_2 gas during sampling.

Red lamps were used in the room during the handling of samples to minimize photodecompositon. Samples were periodically withdrawn from the bottles for chemical analysis and bacterial counts.

When a significant amount of HMX had disappeared, aliquots of the water sample containing the acclimated organisms were inoculated into flasks containing basal salts medium (BSM) and the HMX chemical, with or without yeast extract, or with other additional organic nutrients.

The phosphate-buffered BSM contained, per liter, 1.8 g of K_2HPO_4, 0.2 g of KH_2PO_4, 0.5 g of $(NH_4)_2SO_4$, 0.05 g of NaCl, 0.05 g of $MgSO_4 \cdot 7 H_2O$, 0.01 g of $CaCl_2 \cdot 2 H_2O$, 0.0025 g of $Fe_2SO_4 \cdot 7 H_2O$, and 1 ml of trace elements solution. The trace elements solution contained, per liter, 0.1 g of H_3BO_3, 0.05 g each of $CuSO_4 \cdot 4 H_2O$, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7 H_2O$, Na_2MoO_4, and $CoCl_2 \cdot 6 H_2O$.

Bacterial counts were made from water samples that were serially diluted with sterile phosphate buffer (0.5 g $liter^{-1}$, pH 7), and plated by the pour method, using Difco Plate Count Agar. In the water that contained sediment, the suspension was diluted in 5 ppm Tween 80/phosphate buffer. The counts were made for the plate that showed 30 to 300 colonies after one week of incubation at 25°C, then multiplied by a dilution factor, and expressed as CFU (colony forming units) ml^{-1}.

The biotransformation of HMX was monitored using HPLC methods of analysis. Samples were prepared by combining 0.9 ml of sample with 0.9 µl of internal standard solution (3,5-dinitrotoluene in MeOH). Each sample was then filtered (0.45-µm filter) to remove particulates and bacteria.

The reverse-phase HPLC analysis utilized a Waters radial compression module with a C_{18} RCM cartridge. The mobile phase consisted of A) H_2O and B) 50% methanol/50% acetonitrile. A linear gradient was run from 35 to 55% B in 15 min, with a flow rate of 2.0 ml/min. Detection was by UV at 254 nm. Quantitation was by the internal standard method. A digital integrator was used to determine peak area.

1. Aerobic Biotransformation

The first screening tests for the aerobic and anaerobic biotransformation of HMX were done on Holston River water that had been collected downstream of the HMX wasteline.

The aerobic study was conducted with 2-liter samples containing 4 ppm HMX and (1) river water alone, (2) river water plus 50 ppm sterile yeast extract, and (3) river water plus 1% dry weight of bottom sediment from the HMX wastelines. The initial aerobic bacterial plate count was 2.5×10^5 CFU ml^{-1}.

In the river water alone, the HMX concentration did not decrease after 15 weeks of incubation under aerobic conditions (Figure 5). In the presence of HMX wasteline sediment, there was also no significant aerobic biotransformation. The bacterial count was about 1×10^5 CFU ml^{-1} in water alone and about 3×10^5 CFU ml^{-1} in water with sediment during the experiments.

Unexpectedly, and contrary to the results obtained from the RDX study, HMX was transformed rapidly when small amounts of extra organic nutrient were added. The HMX concentration in the yeast-extract-added water decreased to less than 0.1 ppm in three days of incubation. By HPLC analysis, four metabolites were observed in the chromatographic profile (UV at 254 nm) (Figure 6). We inoculated the microorganisms into

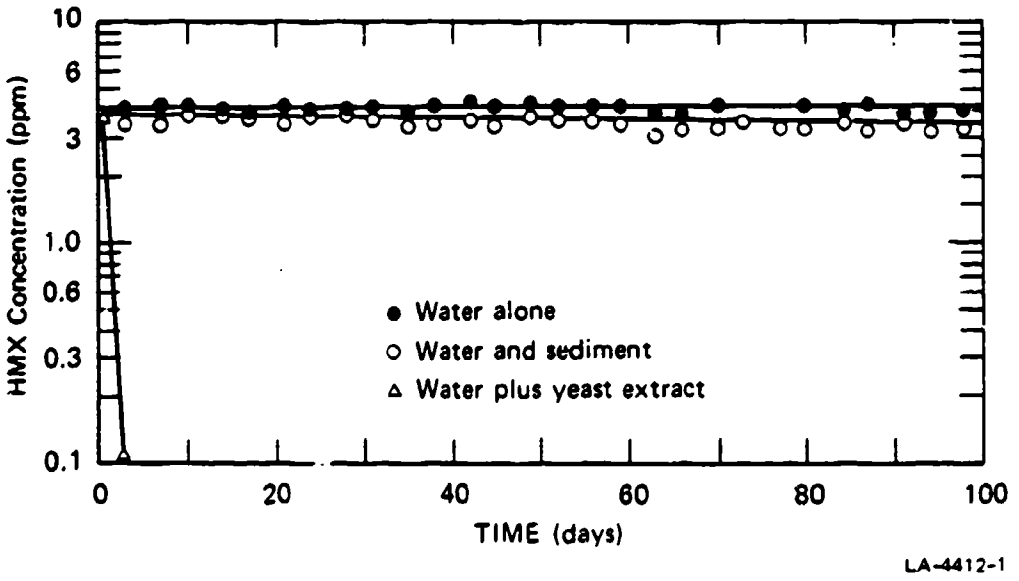


FIGURE 5 HMX AEROBIC BIOTRANSFORMATION IN RIVER WATER

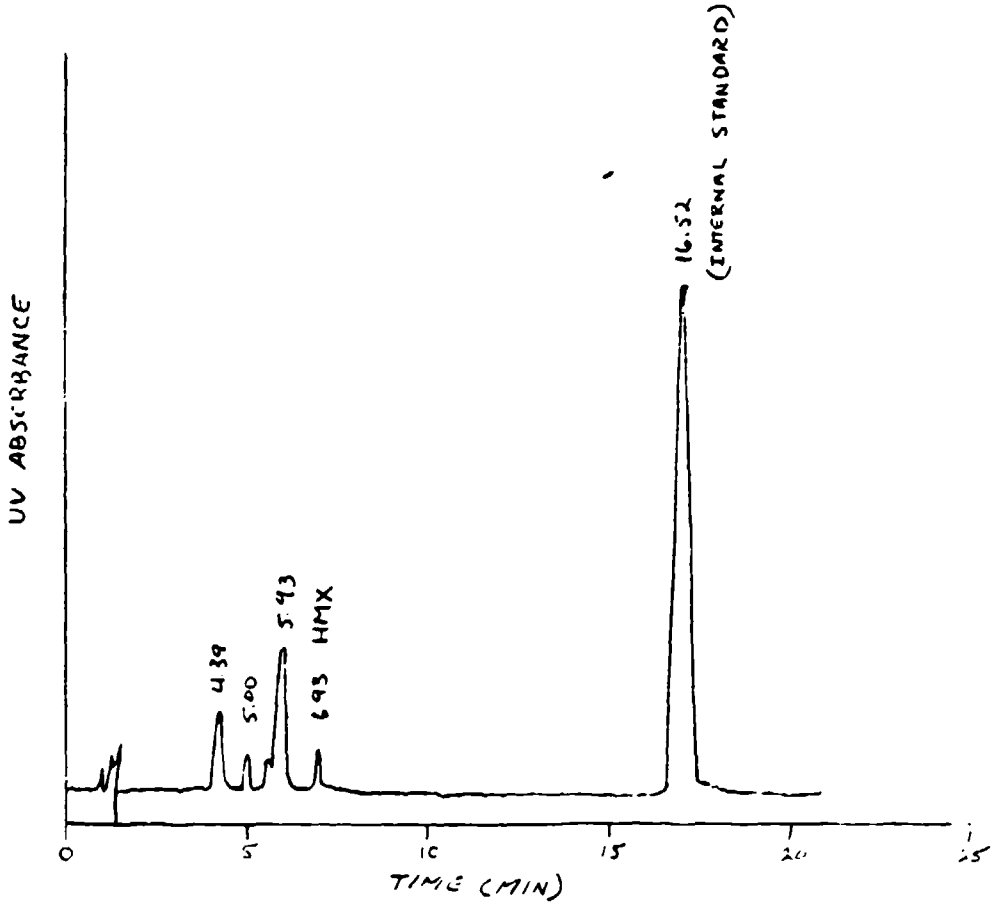


FIGURE 6 - HPLC PROFILE OF THE AEROBIC BIOTRANSFORMATION OF HMX

a 250-ml flask containing 100 ml of BSM, 4 ppm HMX, and 50 ppm yeast extract for the culture enrichment. However, no HMX transformation was observed in 25 days. Another transfer was made from the bottle to the second flask at Day 21; again, no biotransformation was observed. We repeated the original experiment using 600 ml of river water (refrigerated for three weeks), 4 ppm HMX, and 50 ppm yeast extract, but no transformation was observed after 17 days.

The original water containing HMX-degrading aerobic organisms was inoculated into flasks containing (1) 4 ppm HMX and 50 ppm yeast extract in BSM; (2) 4 ppm HMX, 50 ppm glucose, and 10 ppm yeast extract in BSM; and (3) 4 ppm HMX and 50 ppm Difco nutrient broth in BSM. After nine days of incubation, no HMX transformation was observed.

Apparently, microorganisms are present in the freshly collected Holston River water and degrade HMX aerobically after small amounts of yeast extract are added. The transformation could not be repeated with stored river water, and the organisms apparently could not grow in the transferring flasks.

We obtained a fresh water sample from HAAP and repeated the test. The sample was collected from the HMX line wastestream approximately 20 feet below the outflow pipe and was delivered to SRI by overnight Federal Express. HMX was added to the water sample along with 50 ppm of yeast extract or 100 ppm of Difco Brain-Heart Infusion (BHI) medium. After aerobic incubation of the sample, for 2.5 days, the HMX was completely transformed. The organisms were transferred to flasks containing HMX, BSM, and yeast extract or BHI.

In the yeast-extract-added medium, 80% HMX transformation took place in the first transfer flask within 3 days, but no transformation was observed in the second transfer flask or in later transfers.

In BHI-added medium, 90% HMX transformation took place in the first transfer flask within 3 days. The 2nd to 4th transfer flask had 50% HMX transformation (did not decrease thereafter), and no transformation was observed in the 5th and 6th transfer flasks. Transfers were made more

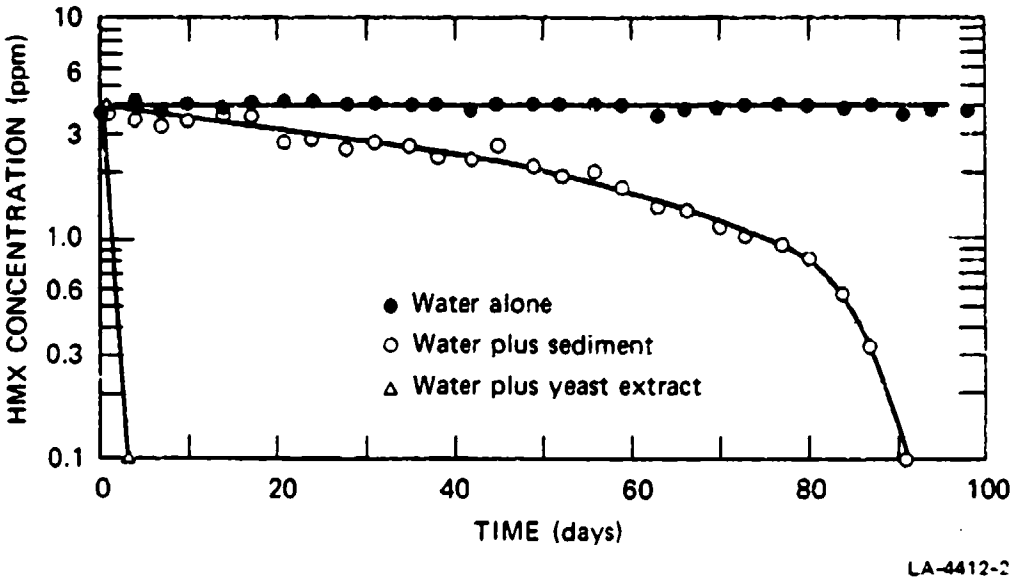


FIGURE 7 HMX ANAEROBIC BIOTRANSFORMATION IN RIVER WATER

than once from each flask and at various times, but still we could not obtain an enriched culture.

HMX in BHI medium was also inoculated with the organisms from the agar plate cultures that were made from the stream water sample when it was received. No HMX transformation was observed in this flask.

The results showed that aerobic HMX biotransformation microorganisms are present in the stream water and in the Holston River near the outfall of the stream. They can grow well with the nutrient of the wastewater and may be biotransforming some of the HMX in the wastewater, but we were unable to obtain an enriched culture under laboratory conditions. The organisms that could aerobically transform HMX came from freshly collected water. These organisms could have died or have been prey for other organisms growing in the laboratory environment.

2. Anaerobic Biotransformation

For the anaerobic studies, 1 liter of each of the waters similar to those used in the aerobic studies (1-3) was placed in a 1-liter Erlenmeyer flask.

In the river water alone, degradation was not significant after 15 weeks of incubation. In the presence of HMX wasteline sediment, HMX was slowly biotransformed. The content was reduced from 4 ppm to 2 ppm after 50 days of incubation and then was further reduced to less than 0.2 ppm (HPLC detection limit by direct aqueous injection) at Day 91 (see Figure 7). The transformation curve is indicative of growth of biotransformation bacteria that accelerate the degradation. It appears that the sediment provides sufficient nutrients to maintain and grow the HMX-biotransforming microorganisms during the anaerobic HMX-transforming period. When this water was inoculated into the medium containing HMX, yeast extract, and BSM, HMX was degraded within one week in the transferring flask, confirming that the transformation is biological.

As in the case of RDX transformation, the HMX biotransformation was more significant in water containing extra organic nutrients (yeast extract). The HMX concentration in anaerobic waters decreased to less than 0.1 ppm in three days. In the anaerobic incubation, one metabolite was observed in the HPLC profile.

When the anaerobic organisms were inoculated into a 125-ml flask containing 100 ml of BSM, 4 ppm HMX, and 50 ppm yeast extract, more than 80% of the HMX was consumed in six days. After continuous transfer of the microorganisms to similar media, we obtained a transferable mixed culture that degraded HMX in three days in yeast-extract-added HMX-BSM.

3. Discussion

As expected from the results of our previous study with RDX (Spanggord, et al. 1980), HMX is relatively persistent in river water without extra nutrient under either aerobic or anaerobic conditions.

Under anaerobic conditions, HMX can be slowly transformed with small amounts of HMX wastestream bottom sediment (1% dry weight). Biotransformation was very fast when 50 ppm of yeast extract was added; HMX was biotransformed to undetectable levels in three days. This result is similar to that observed in RDX biotransformation studies.

Under aerobic conditions, HMX was rapidly biotransformed with freshly collected, yeast-extract-added river water and wastestream water. Unfortunately, we found that this organism(s) was not enriched in the medium containing yeast extract or BHI and BSM. The yeast extract or BHI may be too rich a nutrient for the transforming organisms and the extra nutrient may facilitate growth of other organisms that may phase out or prey on the degradative organisms.

In any case, there are aerobic microorganisms present in the waters that can biotransform HMX when a small amount of organic nutrient is present. Some organic compounds in the HMX wasteline water may provide nutrient for the organisms to grow, maintain population, and perhaps degrade part of HMX in the wastewater.

To further understand the nature of aerobic HMX biotransformation, it is necessary to study (1) the initial organic and inorganic components of HMX wastewater and the use of those components as a supplemental nutrient to grow the organisms, (2) the relationship of the amount of added nutrient to the HMX degradation rate, and then (3) the projection of the biodegradation rate constant under simulated conditions. A study of the biotransformation products is also necessary to provide insight into the degradative mechanism and the overall environmental impact.

B. Biosorption Study

Biosorption studies of HMX on biomass were conducted with mixtures of the following species of gram-positive and gram-negative aquatic bacteria: Azotobacter beijerinckii ATCC 19360, Bacillus cereus ATCC 11778, Escherichia coli ATCC 9637, and Serratia marcescens ATCC 13880. The organisms, except A. beijerinckii, were grown individually in trypticase soy broth at 25°C; A. beijerinckii was grown in ATCC media #16 Azotobacter medium. During the late-logarithmic growth phase the cells were harvested, washed, and resuspended in sterile water. For each species, organism suspensions of equal optical density were mixed together and diluted to a desired concentration. HMX was added to this mixed cell suspension and incubated at 25°C on a reciprocal shaker. The suspension was centrifuged, and the HMX was extracted from the cell pellets and supernatant with methylene chloride. The extract was dried with Na_2SO_4 and the solvent was evaporated under vacuum to dryness. A small amount of methanol, containing internal standard, was added to the extract, which was then analyzed for HMX. The dry weights of the cells were determined after drying in a 90°C oven.

The biosorption partition coefficient (K_B) of HMX between bacteria and water was determined as shown in (Equation 12):

$$K_B = \frac{\mu g \text{ HMX per g dry weight of cells}}{\mu g \text{ HMX per ml in supernatant}} \qquad (12)$$

Preliminary tests showed that HMX was not biotransformed by biosorption microorganisms during the testing period. The preliminary test also showed that the biosorption coefficient was low; therefore, high cell concentrations were used in the study. The results are summarized in Table 9. The average biosorption coefficient of 63 suggests that biosorption will not be an important factor in the fate of HMX in the environment.

Table 9

HMX BIOSORPTION BY BACTERIA

Initial HMX (ppm)	Cell Concentration ($mg\ ml^{-1}$)	Sorption Coefficient (Average ± SD)
1.85	5.9	65 ± 6
0.93	5.9	59 ± 6
0.93	8.9	64 ± 11
	Overall Average:	63 ± 8

VI. RECOMMENDATIONS

On the basis of the previous screening studies, we believe that physical transport processes (adsorption and volatilization) have little impact in controlling the loss and movement of HMX in aquatic environments. Photolysis and biotransformation appear to be the dominant transformation processes and are recommended for detailed study in Phase II.

The photochemical studies should include the determination of the photochemical rate constant over several half-lives and an evaluation of product formation and distribution.

An understanding of the factors that influence the aerobic biotransformation of HMX is necessary to estimate a biotransformation rate constant. Studies should be performed to identify critical nutrients in the HMX wasteline water that support the growth of aerobic organisms and the relationship of these nutrients to the transformation rate of HMX. Products should be identified as part of the overall environmental assessment and as indicators of the potential mechanisms of aerobic biotransformation. We recommend a similar series of studies on the anaerobic organisms to determine pseudo-first-order and second-order biotransformation rate constants.

VII. REFERENCES

Barkley, J.J., Jr. 1977. Determination of the solubility of 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) in water and acetone/water. Personal communication to J. Gareth Pearson and Mark C. Warner.

Campbell, A.N., 1930. An apparatus for the determination of solubility. J. Chem. Soc., London, Part I, pp 179-80.

Douse, J.M.F. 1981. Trace analysis of explosives at the low picogram level of silica capillary column gas: - Liquid chromatography with Electron- Capture Detection. J. Chromatogr. 208, 83-88.

Epstein, S., and C.A. Winkler 1951. Studies of RDX on related compounds. VI. The homogenous hydrolysis of cyclotrimethylenetrinitramine (RDX) and cyclotetranethylenetetranitramine (HMX) in aqueous acetone, and its application to analysis of HMX in RDX. Can. J. Chem. 29, 731-733.

Glover, D. J., and J.C. Hoffsommer 1973. Thin-Layer Chromatographic Analysis of HMX in Water. Bull. Environ. Contamin. and Toxicol., 10, (5), 302-3.

Hoffsommer, J.C., D. A. Kubose, and D.J. Glover 1977. Kinetic Isotope effects and intermediate formation for the aqueous alkaline homogeneous hydrolysis of 1,3,5-triaza-1,3,5-trinitrocyclohexane (RDX). J. Phys. Chem. 81, 380-385.

Jones, W.H. 1954. Mechanism of the homogeneous alkaline decomposition of cyclotrimethylenetrinitramine: Kinetics of consecutive second-and first- order reactions. A polarographic analysis for cyclotrimethylenetrinitramine. J. Am. Chem. Soc. 76, 829-835.

Karickhoff, S.W., D.S. Brown, and T.A. Scott 1974. Sorption of hydrophobic polluntants on natural sediments. Water Res. 13, 241.

Khalifa, S., R.L. Holmstead, and J. E. Casida 1976. Toxaphene degradation by iron (II) protoporphyrine systems. Agri. Food Chem. 24, 277.

Lafeur, A. L., and B. D. Morriseau 1980. Identification of explosives at trace levels by high performance liquid chromatography with a nitrosyl-specific detector. Anal. Chem. 52, 1313-1318.

Liss, P.S., and D. G. Slater 1974. Flux of gases across the air-sea interface. Nature 2-17, 181.

Mackay, D., W.Y. Shiu, and R.P. Sutherland 1979. Determination of air-water Henry's law constants for hydrophobic pollutants. Environ. Sci. Technol. 13, 333-337.

May, W.E., and S.P. Wasik 1978. Determination of the aqueous solubility of polynuclear aromatic hydrocarbons by a coupled column liquid chromatographic technique. Anal. Chem. 50, 175-179.

McCormick, N.G., J. H. Cornell, and A.M. Kaplan 1981. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. Appl. Environ. Microbiol. 42, 817.

Mill, T., D.G. Hendry, and H.R. Richardson 1980. Free-radical oxidants in natural waters. Science 207, 886.

Mill, T., W. R. Mabey, D. C. Bomberger, T. W. Chou, D. G. Hendry, and J. H. Smith 1980. Laboratory Protocols for Evaluating the Fate of Organic Chemicals in Air and Water. Final Report EPA Contract 68-03-2227, SRI International, Menlo Park, CA.

Sikka, H.C. 1980. Environmental Fate for RDX, Progress Report, Syracuse Research Corporation, Syracuse, New York, Contract DAMD 17-77-C-7026.

Smith, J.H., D.C. Bomberger, and D.L. Haynes 1980. Prediction of volatilization rates of high volatility chemicals from natural water bodies. Environ. Sci. Technol. 14, 1332.

Smith. J.H., D.C. Bomberger, and D.L. Haynes. 1981. Volatilization ratio of intermediate and low volatility chemicals from water. Chemosphere 10, 281-289.

Spanggord, R.J., T. Mill, T.W. Chou, W.R. Mabey, J.H. Smith, and S. Lee 1980. Environmental Fate Studies on Certain Munition Wastewater Constituents. Phase II - Laboratory Studies, Final Report. USAMRDC Contract DAMD 17-78-C-8081, SRI International, Menlo Park, CA.

Vouros, P., and B A. Peterson, L. Colwell and B.L. Karger 1977. Analysis of explosives by high performance liquid chromatography and chemical ionization mass spectrometry. Anal. Chem. 49 (7) 1039-1044.

DISTRIBUTION LIST

25 copies	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234
1 copy	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBD-A/Librarian Fort Detrick, Frederick, MD 21701